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Modeling and Inhibitor Design of $Ca^{(2+)}$-Independent Phospholipase A2

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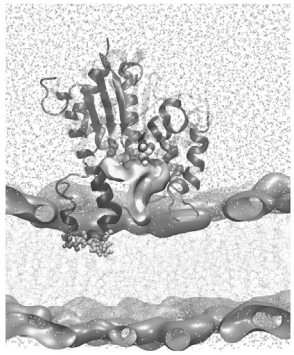
$Ca^{(2+)}$-independent phospholipase A2 (iPLA2) is a water-soluble enzyme that is active in association with lipid membranes. The deregulation of iPLA2 causes uncontrolled inflammation that plays a role in a large number of diseases. The design of selective inhibitors of iPLA2 has been complicated by the absence of any X-ray or NMR information about the enzyme structure. Here we have carried out microsecond MD simulations on homology models of iPLA2 to reach a complete understanding of the dynamic and membrane-related processes associated with the enzyme function and inhibition. The simulations help answer questions that have challenged scientists in this field for decades about the nature of the protein-bilayers complex, the importance of dynamics in the regulation of the active-site pocket, and the detailed mechanism of lipid extraction.



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Membrane Dependence of the Mechanosensitive Channel of Large Conductance

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The mechanosensitive channels of large conductance (MscL) are bacterial membrane proteins that serve as last resort emergency release valves in case of severe osmotic down shock. The channels activate with applied bilayer tension, opening a large (ca. 3 nS) mostly unselective pore. Among the mechanosensitive channels, MscL is the most studied and often used as a model for how proteins sense membrane tension. In addition to sensing bilayer tension MscL channels are influenced by various changes in the bilayer environment. For example, their gating kinetics is shifted by changes in bilayer thickness and lipid head group type; and they can be gated without applied tension by asymmetric addition of lysophosphatidylcholine.We use coarse-grained Martini molecular dynamics model[1,2] in combination with experiments to systematically explore the lipid bilayer influence on MscL function. We characterize the dependence of MscL gating kinetics on bilayer properties by simulating MscL embedded in bilayers of different composition and with systematic addition of straight chain alcohols. Both bilayer bulk properties and local properties/deformation around the proteins are analysed in addition to MscL time to opening after applied tension (ko). Analyses of over a hundred channel opening simulations reveal a short initial lag phase followed by an exponentially distributed channel opening time. In-silico predictions in different channel environments are compared with experimental data determined using reconstituted MscL in a liposomal fluorescent efflux assay. The in-silico model correctly predicts known MscL behaviour, like longer ko in thicker bilayers. Surprisingly, the model predicts longer ko with the addition of alcohols, a finding which was later experimentally confirmed.

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[2] S. Yefimov, E. van der Giessen, P.R. Onck, S.J. Marrink. Biophys. J., 94:2994-3002, 2008.

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Understanding the Special Properties of [NiFeSe] Hydrogenases through the Use of Computational Methodologies

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Hydrogenases are metalloenzymes that catalyze the reversible reaction $H_2 \rightleftarrows 2H^+ + 2e^-$. In order to use H_2 as fuel in a sustainable way, we should learn how to use hydrogenases' unique ability to oxidize and produce H_2 without the need for high temperatures, high over potentials and noble metals.

Two main families of hydrogenases can be distinguished: the [FeFe] and the [NiFe] hydrogenases. Among the last ones, there is a subgroup with outstanding properties - the [NiFeSe] hydrogenases. They have higher activities and are less inhibited by product during H_2 production, and they are more tolerant to the inhibition by O_2 than the standard [NiFe] hydrogenases. However, the structural determinants responsible for these special properties remain unknown. The replacement of the one of the active site cysteines by a selenocysteine, apparently, is not enough to explain the differences between the standard [NiFe] and the [NiFeSe] hydrogenases. In addition, it is also not understood, why some [NiFeSe] hydrogenases can exist in a membrane form and in a soluble form, and why is the membrane form more active when the only difference between them is a lipidic tail for membrane anchoring.

Our aim is to the answer the open questions regarding the special features of [NiFeSe] hydrogenases using computational methodologies such as molecular dynamics and continuum electrostatics. Our first results indicate that the [NiFeSe] hydrogenases have an alternative channel for H_2 diffusion between the protein exterior and the deeply buried active site non-existing in standard [NiFe] hydrogenases. We have also been able to identify differences in the proton transfer pathways between the two types of hydrogenases.

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Structural Insights on the Statherin N-Terminal Binding Domain in the Adsorbed State

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Hard tissues in living organisms form through protein-mediated mechanisms. Although work is being done to deduce many biomineralization processes, a comprehensive understanding of the organic-inorganic interface remains elusive. One reason for this is the difficulty involved in resolving detailed images of biomolecules in the adsorbed state, as opposed to in solution or as crystals. Surface analysis techniques are excellent at measuring general higher-order structural content, in addition to specific geometric features, but still lack the ability to image whole proteins with atomic precision.

A unique partnership emerges from this conundrum. Computer simulation - specifically Molecular Dynamics (MD) - can be used in concert with experiments to provide a more detailed representation of the biomolecule-surface interface. Here we use MD, in conjunction with Parallel Tempering and Metadynamics, to model the first fifteen N-terminal residues of the salivary protein statherin (SN15) in the presence of hydroxyapatite. The SN15 domain lacks any persistent secondary structure in solution, whereas it adopts an α-helical conformation in the adsorbed state. From a converged simulation we show examples of low free energy structures found in the adsorbed state, and compare our own calculated observables to experimental values reported in the literature.

While it is true that other researchers have performed detailed structure prediction calculations on a similar system, this work represents an important step for MD-based biomolecule adsorption simulations. Generally, the two approaches can be thought of in terms of the former being a "top-down" method and the latter a "bottom-up" method. Top-down structure prediction sets a certain number of constraints, and then minimizes a system until it arrives at a best fit. Our bottom-up technique builds a statistical ensemble over time, which ultimately yields a converged dataset that can be understood thermodynamically.

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Understanding the Molecular Mechanisms by which Allosteric Ligands Inhibit the RNA Polymerase from the Hepatitis C Virus

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The Hepatitis C Virus (HCV) infects roughly 170 million individuals worldwide. Currently, there is no cure and available treatments have limited efficacy and sever side-effects. Therefore, new HCV treatments are in high demand. The RNA polymerase (gene product NS5B) from HCV is a validated drug target because of its importance for viral replication. Currently, there are five known allosteric binding sites to which diverse inhibitors can bind. However, the molecular mechanisms that underlie allosteric inhibition are unclear from the available structural and biochemical data. Previously, we employed molecular dynamics (MD) simulations and other computational analyses to characterize the dynamics of free and ligand-bound NS5B to better understand how allosteric inhibitors prevent polymerase function. This information was used to predict which amino acids might be necessary for functional dynamics and/or mediating allosteric inhibition. Mutating residues necessary for functional dynamics should yield a non-functional protein, while mutating residues necessary for allosteric inhibition should yield a functional enzyme even in the presence of inhibitor. Here, we report the results of simulations performed on mutant enzymes to test our predictions. Results from this study will provide a better understanding of the molecular mechanisms of allosteric regulation of NS5B and related polymerases. In addition, this knowledge may facilitate the development of novel inhibitors for NS5B, thus leading to more effective treatments for HCV.